Neolemnane-Type Sesquiterpenoids from a Formosan Soft Coral *Paralemnalia thyrsoides*

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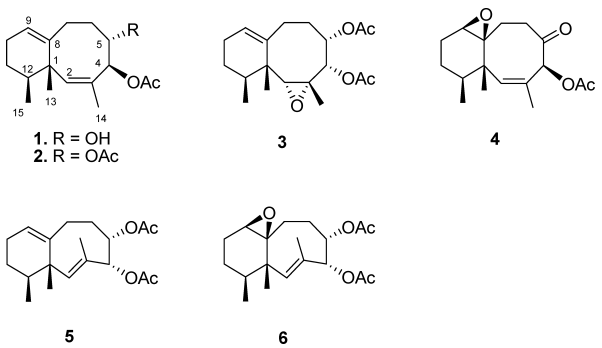
Six new sesquiterpenoids, paralemnolins D—I (1—6), have been isolated from the EtOAc extract of the soft coral *Paralemnalia thyrsoides*. The structures of these metabolites were determined by extensive spectroscopic analysis and by comparison of their spectral data with those of related metabolites. The absolute stereochemistry of these metabolites was established by application of the Mosher's method on 1 and on the basis of the absolute structures of other related compounds previously isolated from the soft corals of the genera *Paralemnalia* and *Lemnalia*. Cytotoxicity of these metabolites toward a limited panel of cancer cell lines also is reported.

Key words *Paralemnalia thyrsoides*; soft coral; sesquiterpenoid; paralemnolin

Soft corals belonging to the genera *Paralemnalia*[1—4] and *Lemnalia*[5—12] have been found to be a rich source of neolemnane and nardosinane sesquiterpenoids. Our previous investigation on the chemical constituents of a soft coral *Paralemnalia thyrsoides* has resulted in the isolation of three terpenoids, paralemnolins A—C.[13] Continuing study on searching new metabolites of this soft coral again afforded six new metabolites paralemnolins D—I (**1**—**6**) (neolemnanoids). We describe herein the isolation, structure elucidation and biological activity of these compounds.

Results and Discussion

Paralemnolin D (**1**) was isolated as a colorless oil, $[\alpha]_D^{25}$ +67° (c=1.32, $CHCl_3$). Its HR-ESI-MS exhibited a pseudomolecular ion peak at m/z 301.1781 $[M+Na]^+$ (Calcd for 301.1780), corresponding to the molecular formula of $C_{17}H_{26}O_3$. Thus, five degrees of unsaturation were deduced. The ^{13}C-NMR and DEPT spectra displayed 17 carbon signals, including four methyls, four methylenes, five methines, and four quaternary carbons. The NMR signals [δ_H 2.10 (3H, s); δ_C 170.8 (C) and 20.9 (CH_3)] and IR absorptions at 3504 and 1730 cm^{-1}, together with the observation of two oxygen-bearing carbon resonances [δ 72.1 (CH) and 76.3 (CH)] in the ^{13}C-NMR spectrum, indicated the presence of an acetyl and a hydroxy group. Furthermore, two trisubstituted double bonds [δ 125.7 (CH), 142.1 (C), 138.3 (CH), 129.6 (C)] were assigned from the ^{13}C-NMR and DEPT spectra of **1**. The above functionalities revealed that **1** is a bicyclic compound. The skeleton of **1** was established by extensive 2D NMR analysis (^{1}H–^{1}H COSY, HMQC, and HMBC). To establish the proton sequences in **1**, the ^{1}H–^{1}H COSY spectrum was used to reveal the connectivity of H-4/H-5, H-5/H_2-6, H_2-6/H_2-7, H-9/H_2-10, and H_2-10/H_2-11 (Fig. 1). The vinyl methyl [δ 1.57 (3H, d, J=1.2 Hz)] attached at C-3 was confirmed by the HMBC correlations from H_3-14 to C-2, C-3 and C-4. The methyl group attached at C-1 and the connectivities from C-7 to C-8 and C-8 to C-9 were deduced from the following HMBC correlations: H_3-13/C-1, C-2, C-8, C-12 and H_2-7/C-8, C-9. Moreover, a methyl [δ 0.87 (3H, d, J=6.6 Hz)] attached at C-12 was confirmed by the HMBC correlations from H_3-15 to C-1, C-11, and C-12. The placement of the acetyl group at C-4 was confirmed by the HMBC correlation of H-4 (δ 6.49) with C-3 and the carbonyl group of ester. Thus, the hydroxyl group should be positioned at C-5, and the planar structure of **1** was established. The relative stereochemistry and detailed ^{1}H-NMR data assignment of **1** were elucidated from the NOE correlations observed in a NOESY experiment (Fig. 2). The Z geometry of the 2,3-double bond was established by an NOE interaction between H-2 and H_3-14. Also, H_3-13 was found to show NOE interactions



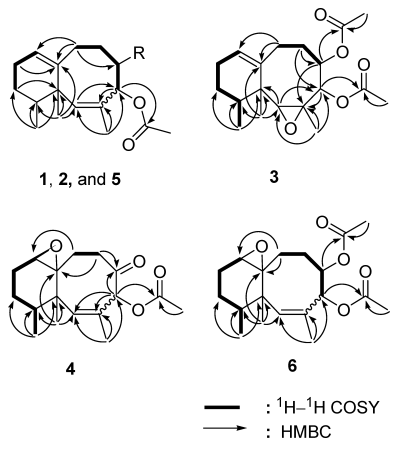


Fig. 1. Key ^{1}H–^{1}H COSY and HMBC Correlations of **1**—**6**

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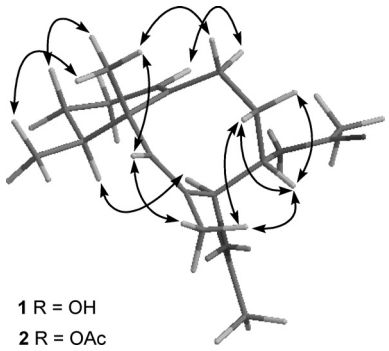


Fig. 2. Selective NOESY Correlations of **1** and **2**

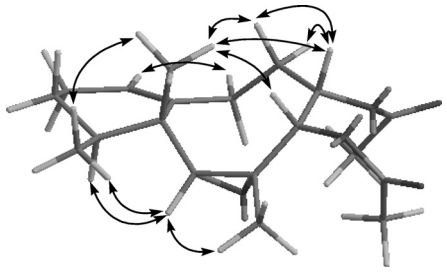


Fig. 3. Selective NOESY Correlations of **3**

with H-2, H_3-15 and one proton (δ 2.31, m) of H_2-7, but not with H-12. H_3-14 also showed an NOE correlation with H-5 instead of H-4, while no NOE correlation could be observed between H-4 and H-5, suggesting that H_3-15, H_3-13, and H-5 should be positioned on the same face and were arbitrarily assigned as β-oriented protons. Moreover, the NOE interaction between H-4 and H-12 suggested that both H-4 and H-12 should be positioned on the α face. Thus, the structure of **1** was established unambiguously.

Paralemnolin E (**2**) was obtained as a white powder, $[\alpha]_D^{25}$ +68° (c=0.68, $CHCl_3$). The molecular formula of **2**, $C_{19}H_{28}O_4$, was established by HR-ESI-MS, requiring six degrees of unsaturation. The IR spectrum of **2** showed the presence of ester groups (ν_{max} 1739 cm^{-1}). The ^{13}C-NMR and DEPT spectra of **2** showed signals of 19 carbons (Table 2), including two ester carbonyls [δ 170.3 (C), 170.8 (C)], and two trisubstituted double bonds [δ 126.4 (CH), 141.0 (C), 138.8 (CH), 129.0 (C)]. The above functionalities revealed that **2** is a bicyclic compound. The NMR spectral data of **2** were analogous to those of **1** with the exception that the resonance of hydroxyl methine in **1** was replaced by that of an acetyl methine in **2**, which was further confirmed by the assistance of the HMBC and ^{1}H–^{1}H COSY spectra as shown in Fig. 1. The relative stereochemistry of **2** was found to be the same as that of **1**, on the basis of the observed NOE correlations between related protons (Fig. 2).

Paralemnolin F (**3**) was obtained as a colorless oil, $[\alpha]_D^{25}$ −6° (c=1.28, $CHCl_3$). According to the HR-ESI-MS (*m/z* 359.1835, $[M+Na]^+$) and ^{13}C-NMR spectral data, its molecular formula was established as $C_{19}H_{28}O_5$, thus indicating six degrees of unsaturation. The IR spectrum of **3** showed the presence of ester functionality (ν_{max} 1745 cm^{-1}) and the absence of hydroxyl group. The ^{13}C- and ^{1}H-NMR spectra revealed the presence of two acetyl groups [δ_H 2.09 (3H, s), 2.07 (3H, s); δ_C 170.0 (C), 169.9 (C), 21.1 (CH_3), 20.9 (CH_3)], one double bond [δ_C 127.8 (CH), 140.4 (C)]. A trisubstituted epoxide was confirmed from the resonances of two oxygen-bearing carbons at δ_C 58.7 (C) and 71.8 (CH), the latter correlating with a proton at δ_H 2.62 (s) in the HMQC spectrum. Comparison of the NMR spectral data of compounds **1**—**3** revealed that the C-2–C-3 double bond of **1** and **2**, was oxidized as an epoxide in compound **3**. The location of the epoxide was confirmed by the HMBC correlations from H_3-14 to C-2, C-3, and C-4 (Fig. 1). The same as that for compound **2**, the locations of two acetyl groups at C-4 and C-5 were revealed by the relevant HMBC correlations, as shown in Fig. 1. Thus, the planar structure of **3** was established. The relative stereochemistry of **3** was deduced by the key NOE interactions (Fig. 3). From the NOESY spectrum of **3**, H_3-13 was found to show NOE interactions with H-4, H-5, H_3-15 and one proton (δ 1.97, m) of H_2-6, but not with H-2 and H-12, revealing the β-orientation of these protons. Furthermore, H-2 (δ 2.62, s) exhibited NOE interactions with H_3-14, H_3-15 and H-12, suggesting the β-orientation of H-2 and H_3-14, as revealed by the molecular model in Fig. 3. Thus, the structure of **3** was established unambiguously.

Compound **4** was obtained as a colorless oil. The HR-ESI-MS of **1** exhibited a pseudomolecular ion $[M+Na]^+$ peak at *m/z* 315.1573 and established a molecular formula of $C_{17}H_{24}O_4$, implying six degrees of unsaturation. The ^{13}C-NMR and DEPT spectra of **4** displayed 17 carbon signals: four methyls, four methylenes, four methines, and five quaternary carbons. From the ^{1}H- and ^{13}C-NMR spectra of **4**, the resonances at δ_C 170.3 (C), 20.6 (CH_3) and δ_H 2.17 (s) disclosed the presence of an acetyl group. Furthermore, one ketone (δ_C 201.4), one trisubstituted epoxide [δ_C 59.6 (CH) 66.1 (C)], and one trisubstituted double bond [δ_C 142.0 (CH) 126.6 (C)] were also found in the ^{13}C-NMR spectrum. The above functionalities accounted for four of the six degrees of unsaturation, suggesting a bicyclic structure for **4**. Comparison of the NMR spectral data of **4** with those of **1**—**3** disclosed that they should possess the same carbon skeleton. The gross structure of **4** was established by the careful inspection of the 2D NMR (^{1}H–^{1}H COSY and HMBC) correlations as illustrated in Fig. 1.

The presence of NOE interactions between H-2 and H_3-14 suggested the *Z* geometry of the 2,3-double bond. The β-oriented H_3-13 was found to show NOE correlations with H_3-15, H-2 and one proton of H_2-7 (δ 2.40, m), but not with H-12, suggesting the β-orientation of this C-7 attached proton, H_3-13, and H_3-15. Moreover, H-4 was found to show NOE interactions with H-12 and the α-oriented proton at C-10 (δ 1.94, m), and H-9 also showed an NOE interaction with the α-oriented proton of H_2-7 (δ 1.57, m), revealing the α-positions of H-4, H-9 and H-12. Thus, the structure of **4** was established unambiguously.

Compound **5** was found to possess the same molecular formula as that of **2** ($C_{19}H_{28}O_4$), as revealed from HR-ESI-MS. The above result, together with the comparison of the 2D NMR spectral data of **5** with those of **2**, disclosed that both compounds had similar structures. However, due the overlap of proton signals in the ^{1}H-NMR spectrum of **5**, observed in $CDCl_3$ solution, the NOESY spectrum was recorded in pyridine-d_5. In this spectrum H_3-13 was found to show NOE correlations with H_3-15 and H_3-14, but not with H-12 and H-2. Moreover, NOE correlations were observed

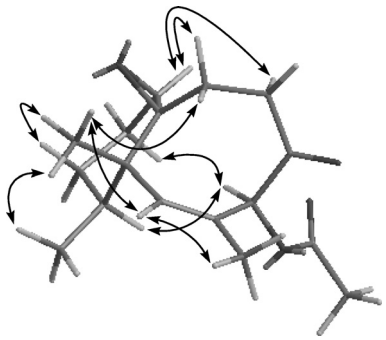


Fig. 4. Selective NOESY Correlations of **4**

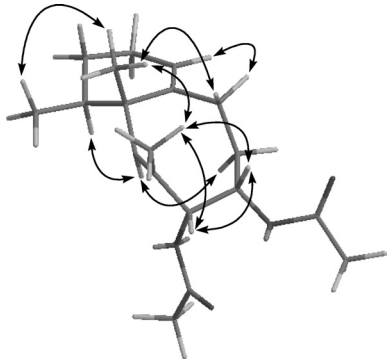


Fig. 5. Selective NOESY Correlations of **5**

Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**6**[a)]

H#	**1**	**2**	**3**	**4**	**5**	**6**
2	5.21 t (2.0)[b)]	5.25 t (2.5)[b)]	2.62 s	5.73 d (1.5)[b)]	5.26 d (1.2)[b)]	5.57 br d (1.2)[b)]
4	6.49 d (9.3)	6.73 d (9.3)	5.30 br s	6.18 s	5.23 d (3.0)	5.25 d (3.0)
5	3.93 m	5.11 ddd (9.6, 2.7, 4.5)	5.27 m		4.90 ddd (9.6, 3.0, 3.3)	4.84 ddd (11.1, 3.0,3.0)
6	β 1.73 m	β 1.80 m	α 1.86 m	2.53 t (1.5)	1.71 m	β 1.57 ddd (11.1, 10.8, 2.1)
	α 2.08 m	α 2.04 m	β 1.97 m			α 1.94 m
7	β 2.31 m	β 2.30 m	α 2.15 m	α 1.57 m	β 1.70 m	α 1.10 m
	α 2.41 m	α 2.33 m	β 2.42 m	β 2.40 m	α 2.20 m	β 1.91 m
9	5.62 dd (3.6, 3.6)	5.64 dd (1.5, 1.2)	5.58 dd (3.6, 3.8)	3.47 d (2.4)	5.38 dd (3.6, 3.6)	3.0 br s
10	2.03 m	2.08 m	2.07 m	α 1.94 m	2.05 m	α 1.82 m
				β 2.15 m		β 2.05 m
11	β 1.41 m	β 1.42 m	1.50 m	α 1.18 m	1.45 m	α 1.08 m
	α 1.79 m	α 1.85 m		β 1.42 m		β 1.33 m
12	1.77 m	1.73 m	1.73 m	1.55 m	1.71 m	1.44 m
13	1.02 s	1.03 s	0.98 s	1.12 s	1.13 s	1.14 s
14	1.57 d (1.2)	1.58 s	1.48 s	1.69 d (4.8)	1.97 d (1.5)	2.0 s
15	0.87 d (6.6)	0.87 d (6.7)	0.97 d (6.9)	0.85 d (6.9)	0.89 d (6.9)	0.78 d (6.9)
OAc	2.10 s	2.02 s	2.09 s	2.17 s	2.00 s	1.99 s
OAc		2.07 s	2.07 s		2.13 s	2.14 s

a) Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. *b*) *J* values (in Hz) in parentheses.

between H_3-14 and H-4, and H_3-14 and H-5. Thus, H_3-13, H_3-15, H-4, and H-5 were assigned as β-protons. The absence of NOE correlation between H_3-14 and H-2 suggested the *E* geometry of 2,3-double bond. By analysis of these NOE interactions and a molecular model as shown in Fig. 5, the relative structure as **5** was confirmed.

Paralemnolin I (**6**) possesses a molecular formula of $C_{19}H_{28}O_5$ as revealed by its HR-ESI-MS and NMR spectral data (Tables 1, 2). Comparison of the ^{13}C-NMR spectral data of **6** with those of **1**—**5** (Table 2) indicated that these compounds possess similar carbon skeleton. Inspection of the ^{13}C-NMR spectral data of **6** allowed the assignment of a 2,3-double bond [δ 130.6 (C) and 136.7 (CH)] and 8,9-epoxide [δ 68.3 (C), 60.7 (CH)]. Two acetyl groups attached at C-4 and C-5 were confirmed by the HMBC correlations from H_3-14 to C-2, C-3 and C-4, and the COSY correlation between H-4 (δ 5.25) and H-5 (δ 4.84) (Fig. 1). The relative stereochemistry was deduced by the assistance of the NOE correlations observed in a NOESY experiment (Fig. 6). The *E* geometry was determined due to the absence of the NOE correlation between H_3-14 and H-2. In the NOESY spectrum of **6**, the β-oriented H_3-13 was found to show NOE correlations with H_3-15, H_3-14 and the β-oriented proton of H_2-7 (δ 1.91, m), but not with H-12. Moreover, NOE correlations were observed between H_3-14 and H-4, H_3-14 and H-5, and H-4 and H-5. Thus, the β-orientation of H_3-15, H-4 and H-5 was suggested. Furthermore, H-2 was found to show an NOE correlation with H-12, while the α-oriented proton of H_2-7 (δ

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**6**[a)]

C#	**1**	**2**	**3**	**4**	**5**	**6**
1	43.0	43.0	40.9	42.2	49.9	47.2
2	138.3	138.8	71.8	142.0	135.6	136.7
3	129.6	129.0	58.7	126.6	129.2	130.6
4	76.3	72.5	73.6	77.2	77.6	76.3
5	72.1	74.0	71.9	201.4	77.2	75.3
6	31.3	28.5	30.2	40.8	31.7	26.5
7	30.3	31.0	29.8	32.7	32.0	35.9
8	142.1	141.0	140.4	66.1	147.7	68.3
9	125.7	126.4	127.8	59.6	123.9	60.7
10	23.4	23.2	25.3	25.6	26.0	26.7
11	26.3	26.2	25.9	24.2	24.8	22.4
12	38.2	38.1	42.8	40.2	39.4	41.4
13	23.1	23.1	17.0	18.6	17.3	14.2
14	20.1	20.1	20.7	17.6	18.2	18.1
15	15.1	14.9	16.2	17.1	18.3	17.4
OAc	170.8	170.3	170.0	170.3	170.1	170.1
	20.9	20.8	20.9	20.6	21.1	21.0
OAc		170.8	169.9		169.9	169.7
		21.2	21.1		21.2	21.2

a) Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C.

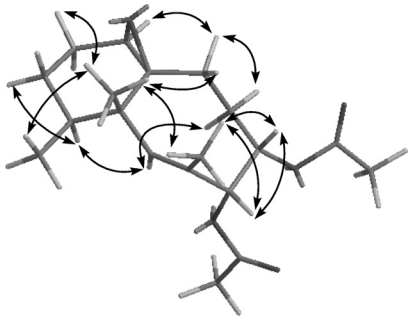
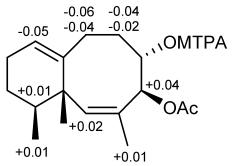


Fig. 6. Selective NOESY Correlations of **6**



1a : *S*-MTPA ester

1b : *R*-MTPA ester

$\Delta\delta=\delta(S)-\delta(R)$ (ppm)

Fig. 7. ^{1}H-NMR Chemical Shift Differences [$\delta(S)$-MTPA ester–$\delta(R)$ MTPA ester]

1.10, 1H, m) showed NOE response with H-9, suggesting that H-12 and H-9 should be position on the α face.

The absolute stereochemistry of paralemnolin D (**1**) was determined by application of the Mosher's method,[14] which led to the assignment of *S*-configuration at C-5 (Fig. 7). Therefore, the absolute structure of **1** was determined as shown in formula **1**. The absolute stereochemistries of several nardosinanoids isolated from the soft corals of the genera *Paralemnalia* and *Lemnalia* also had been determined.[1,4,7—13] On the basis of the above results and biogenic consideration, the absolute configurations at C-1 and C-12 of neolemnanoids were considered to be identical as illustrated in formula **1**—**6**. Thus, the absolute structures of **1**—**6** were established.

All metabolites have been submitted for cytotoxicity evaluation toward cancer cell lines, including Hepa59T/VGH (human liver carcinoma), KB (human oral epidermoid carcinoma), Hela (human cervical epitheloid carcinoma), and Daoy (human medulloblastoma). The result showed that compound **2** exhibited moderated cytotoxicity toward Daoy cancer cell line with the ED_{50} values of 17.5 μM. Other compounds did not show inhibitory activity against the growth of the above four cancer cell lines.

Experimental

General Experimental Procedures Melting points were determined using a Fisher–Johns melting point apparatus and were uncorrected. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. NMR spectra were recorded on a Varian Mercury-Plus 300 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C in $CDCl_3$ using TMS as internal standard. LR-ESI-MS and HR-ESI-MS were obtained on a Burker APEX II Mass spectrometer. Silica gel (Merck, 230—400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10AT apparatus equipped with the Merck Hibar Si-60 column (250×21 mm, 7 μm) or Merck Hibar RP-18e column (250×10 mm, 5 μm).

Animal Material The soft coral *Paralemnalia thyrsoides* was collected by hand using scuba at Green Island, which is located off the southeast coast of Taiwan, in July 2004, at a depth of 15 m, and was stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University (specimen no. GIPT-401).

Extraction and Isolation The soft coral (1.8 kg fresh wt) was collected and freeze-dried. The freeze-dried organism was minced and extracted exhaustively with EtOAc. The combined organic extract was evaporated to give a dark brown residue (33.0 g), which was chromatographed on a silica-gel column using solvents of increasing polarity from *n*-hexane to EtOAc to get fraction 1—20. Fraction 10 eluted with *n*-hexane–EtOAc (10 : 1) was chromatographed by reverse phase HPLC using acetone–H_2O (3 : 1), and were further purified by normal phase HPLC, eluting with *n*-hexane–EtOAc (25 : 1), to afford compounds **2** (11.3 mg) and **4** (2.1 mg). Fraction 13 eluted with *n*-hexane–EtOAc (5 : 2) were subjected to repeated normal phase HPLC column chromatography using *n*-hexane–EtOAc (8 : 1) to yield compounds **6** (50 mg). Compounds **3** (3.3 mg) were obtained from fraction 13 by reverse phase HPLC using acetonitrile–H_2O (2 : 1). Fraction 16 eluted with *n*-hexane–EtOAc (1 : 1) was purified by normal phase HPLC using *n*-hexane–EtOAc (5 : 1) to afford compound **5** (16.2 mg). Fraction 17 eluted with *n*-hexane–EtOAc (1 : 2) was chromatographed by normal phase HPLC using *n*-hexane–EtOAc (5 : 1) and followed by reverse phase HPLC using acetonitrile–H_2O (5 : 1) to afford compound **1** (13.7 mg).

Paralemnolin D (**1**): Colorless oil; $[\alpha]_D^{25}$ +67° (c=1.32, $CHCl_3$); IR (KBr) ν_{max} 3504, 1730, 1653 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z*: 301 $[M+Na]^+$; HR-ESI-MS *m/z*: 301.1781 $[M+Na]^+$ (Calcd for $C_{17}H_{26}O_3Na$, 301.1780).

Paralemnolin E (**2**): White powder; mp 61—62 °C; $[\alpha]_D^{25}$ +68° (c=0.68, $CHCl_3$); IR (KBr) ν_{max} 1739, 1373, 1240 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z*: 343 $[M+Na]^+$; HR-ESI-MS *m/z*: 343.1885 $[M+Na]^+$ (Calcd for $C_{19}H_{28}O_4Na$, 343.1885).

Paralemnolin F (**3**): Colorless oil; $[\alpha]_D^{25}$ −6° (c=1.28, $CHCl_3$); IR (KBr) ν_{max} 1745, 1371, 1244 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z*: 359 $[M+Na]^+$; HR-ESI-MS *m/z*: 359.1835 $[M+Na]^+$ (Calcd for $C_{19}H_{28}O_5Na$, 359.1834).

Paralemnolin G (**4**): Colorless oil; $[\alpha]_D^{25}$ +311° (c=0.84, $CHCl_3$); IR (KBr) ν_{max} 1743, 1726, 1381 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z*: 315 $[M+Na]^+$; HR-ESI-MS *m/z*: 315.1573 $[M+Na]^+$ (Calcd for $C_{17}H_{24}O_4Na$, 315.1572).

Paralemnolin H (**5**): Colorless oil; $[\alpha]_D^{25}$ −90° (c=1.42, $CHCl_3$); IR (KBr) ν_{max} 1741, 1637, 1244 cm^{-1}; ^{1}H- and ^{13}C-NMR data (in $CDCl_3$), see Tables 1 and 2; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ: 5.63 (1H, d, J=3.0 Hz, H-4), 5.52 (1H, d, H-2), 5.33 (1H, dd, J=4.2, 3.0 Hz, H-9), 5.13 (1H, overlapped with H_2O in pyridine-d_5, H-5), 2.22 (1H, m, H-7a), 2.09 (3H, s, OAc), 1.98 (3H, s, OAc), 1.94 (2H, m, H-10), 1.91 (3H, d, J=1.2 Hz, H-14), 1.88 (2H, m, H-6), 1.74 (1H, m, H-7b), 1.66 (1H, m, H-12), 1.31 (2H, m, H-11), 1.06 (3H, s, H-13), 0.81 (3H, d, J=6.9 Hz, H-15); ^{13}C-NMR (pyridine-d_5, 75 MHz) δ: 170.7 (CH_3, OAc), 169.9 (CH_3, OAc), 148.0 (C, C-8), 135.2 (CH, C-2), 130.2 (C, C-3), 124.1 (CH, C-9), 77.6 (CH, C-4), 77.5 (CH, C-5), 50.2 (C, C-1), 39.6 (CH, C-12), 32.3 (CH_2, C-7), 32.0 (CH_2, C-6), 26.2 (CH_2, C-10), 25.0 (CH_2, C-11), 21.0×2 (CH_3, 2×OAc), 18.4 (CH_3, C-15), 18.2 (CH_3, C-14), 17.4 (CH_3, C-13); ESI-MS *m/z*: 343 $[M+Na]^+$; HR-ESI-MS *m/z*: 343.1883 $[M+Na]^+$ (Calcd for $C_{19}H_{28}O_4Na$, 343.1885).

Paralemnolin I (**6**): Colorless oil; $[\alpha]_D^{25}$ −210° (c=1.62, $CHCl_3$); IR (KBr) ν_{max} 1743, 1375, 1228 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z*: 359 $[M+Na]^+$; HR-ESI-MS *m/z*: 359.1835 $[M+Na]^+$ (Calcd for $C_{19}H_{28}O_5Na$, 359.1834).

(*R*)- and (*S*)-MTPA Esters of 1 To a solution of compound **1** (1.0 mg) in prydine (0.5 ml) was added (−)-MTPA chloride (2.3 μl) at room temperature for 4—5 h. The reaction mixture was concentrated to dryness under reduced pressure and purified by a short silica gel column with EtOAc/*n*-hexane (1 : 5) to give (*S*)-MTPA ester **1a** (0.9 mg). The (*R*)-MTPA ester **1b** (0.8 mg) was prepared from (+)-MTPA chloride by the same method. Selected $\Delta\delta$ values were shown in Fig. 3.

Cytotoxicity Testing Cancer Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds **1**—**6** were performed using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method.[15,16]

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